

Appl. No. : 10/801,931
Filed : March 15, 2004

AMENDMENTS TO THE CLAIMS

1. (Currently amended) A method for purifying one or more selected cells in a population, comprising:

(a) contacting a plurality of cells immobilized in proximity to a capture matrix, ~~the capture matrix capable of localizing~~ that localizes a product secreted by one or more of the cells; with an agent that selectively binds to the product, wherein the agent ~~capable of generating~~ generates a signal detectable as a property of light;

(b) illuminating a population of the cells, the population contained in a frame;

(c) detecting two or more properties of light directed from the frame, wherein a first property of light identifies substantially all cells of the population, and ~~the~~ a second property of light identifies product localized to the capture matrix;

(d) locating ~~(i)~~ substantially all cells of the population with reference to the detected first property of light, ~~and (ii)~~;

(e) selecting one or more ~~selected~~ cells with reference to the detected second property of light; and

[[~~(e)~~]](f) irradiating the one or more non-selected cells, wherein each the one or more non-selected cells receives a substantially lethal dose of radiation,

whereby one or more selected cells having a desired product secretion profile are purified.

2. (Currently amended) The method of claim 1, wherein the product is selected from the group consisting of a polypeptide, an antibody, an antibody fragment, a cytokine, a growth factor, an enzyme, a hormone, a neurotransmitter, a signaling molecule, and a therapeutic protein.

3. (Currently amended) The method of claim 1, wherein the capture matrix comprises a substance selected from the group consisting of Protein G, Protein A, an antibody, an antibody fragment, an aptamer, and a ligand for the product.

4. (Original) The method of claim 1, wherein the capture matrix comprises a gel.

Appl. No. : 10/801,931
Filed : March 15, 2004

5. (Currently amended) The method of claim 1, further comprising the step of ~~[[[(f)]]~~ (g) illuminating a further population of the cells, the further population contained in a further frame, and repeating steps (c) through ~~[[[(e)]]~~(f).

6. (Currently amended) The method of claim 1 ~~[[or 5]]~~, wherein the one or more selected cells are cells that produce a high level of product relative to other cells of the population.

7. (Currently amended) The method of claim 1 ~~[[or 5]]~~, wherein the one or more selected cells are cells that produce a low level of product relative to other cells of the population.

8. (Currently amended) The method of claim 1 ~~[[or 5]]~~, wherein the one or more selected cells are cells that produce an undetectable level of product.

9. (Currently amended) The method of claim 5, further comprising repeating step ~~[[[(f)]]~~(g) followed by steps (c) through ~~[[[(e)]]~~(f) until substantially all of the non-selected cells in the plurality of cells receive a substantially lethal dose of radiation.

10. (Original) The method of claim 9, wherein the one or more selected cells are cells that produce a high level of product relative to other cells of the plurality.

11. (Original) The method of claim 9, wherein the one or more selected cells are cells that produce a low level of product relative to other cells of the plurality.

12. (Original) The method of claim 9, wherein the one or more selected cells are cells that produce an undetectable level of product.

13. (Original) The method of claim 1, further comprising allowing the purified one or more selected cells to proliferate.

14. (Original) The method of claim 1, wherein steps (b) through ~~[[[(e)]]~~(f) are automated.

15. (Currently amended) The method of any of claims 1, 5 or 9, wherein the frame is a portion of a field-of-view, the field-of-view having an area ~~selected from the group of greater than 5 mm², greater than 10 mm², greater than 20 mm², greater than 40 mm², greater than 80 mm², and greater than 160 mm².~~

Appl. No. : 10/801,931
Filed : March 15, 2004

16. (Currently amended) The method of claim 1, wherein the agent that selectively binds to the product is selected from the group consisting of an antibody, an antibody fragment, an aptamer, a substrate, and a ligand.

17. (Original) The method of claim 1, wherein the one or more selected cells are identified with reference to a signal value corresponding to an amount of product localized to the capture matrix in the vicinity of the one or more selected cells.

18. (Original) The method of claim 1, wherein the substantially lethal dose of radiation is delivered to each non-selected cell in one or more radiation pulses.

19. (Currently amended) The method of claim 1, wherein the substantially lethal dose of radiation has an energy density selected from the group consisting of greater than 0.1 J/cm², greater than 0.3 J/cm², greater than 1 J/cm², greater than 3 J/cm², greater than 10 J/cm², greater than 30 J/cm², and greater than 100 J/cm².

20. (Currently amended) The method of claim 1, wherein the substantially lethal dose of radiation has an irradiance selected from the group consisting of greater than 10⁷ W/cm², greater than 10⁸ W/cm², greater than 10⁹ W/cm², greater than 10¹⁰ W/cm², and greater than 10¹¹ W/cm².

21. (Currently amended) The method of claim 1, wherein the radiation comprises an electromagnetic radiation having a wavelength selected from the group consisting of between 200 and 400 nm, between 400 and 760 nm, and between 760 and 3000 nm.

22. (Original) The method of claim 1, wherein prior to illumination, the plurality of cells is contacted with a reagent that selectively binds to substantially all of the cells, the reagent capable of generating a signal detectable as a first property of light.

23. (Currently amended) A method for purifying one or more selected cells in a population, comprising:

(a) illuminating a population of cells in a frame, wherein the illuminated cells are contained in a plurality of cells immobilized in proximity to a capture matrix, wherein the capture matrix ~~capable of localizing~~ localizes a product secreted by one or more of the cells;

Appl. No. : 10/801,931
Filed : March 15, 2004

(b) detecting two or more properties of light directed from the frame, wherein a first property of light identifies substantially all cells of the population, and wherein a second property of light identifies product localized to the capture matrix;

(c) locating (i) substantially all cells of the population with reference to the detected first property of light; ~~and (ii)~~

(d) selecting one or more selected cells with reference to the detected second property of light; ~~and~~

[[~~(d)~~]](e) irradiating the one or more non-selected cells, wherein each of the one or more non-selected cells receives a substantially lethal dose of radiation,

whereby one or more selected cells having a desired product secretion profile are purified.

24. (Currently amended) The method of claim 23, wherein the product is selected from the group consisting of a polypeptide, an antibody, an antibody fragment, a cytokine, a growth factor, an enzyme, a hormone, a neurotransmitter, a signaling molecule, and a therapeutic protein.

25. (Currently amended) The method of claim 23; wherein the capture matrix comprises a substance selected from the group consisting of Protein G, Protein A, an antibody, an antibody fragment, an aptamer, and a ligand for the product.

26. (Original) The method of claim 23, wherein the capture matrix comprises a gel.

27. (Currently amended) The method of claim 23, further comprising the step of ~~[[~~(e)~~]]~~(f) illuminating a further population of the cells, the further population contained in a further frame, and repeating steps (b) through ~~[[~~(d)~~]]~~(e).

28. (Currently amended) The method of claim 23 ~~or 27~~, wherein the one or more selected cells are cells that produce a high level of product relative to other cells of the population.

29. (Currently amended) The method of claim 23 ~~or 27~~, wherein the one or more selected cells are cells that produce a low level of product relative to other cells of the population.

Appl. No. : 10/801,931
Filed : March 15, 2004

30. (Currently amended) The method of claim 23 ~~or 27~~, wherein the one or more selected cells are cells that produce an undetectable level of product.

31. (Currently amended) The method of claim 27, further comprising repeating step ~~[(e)]~~(f) followed by steps (b) through ~~[(d)]~~(e) until substantially all of the non-selected cells in the plurality of cells receive a substantially lethal dose of radiation.

32. (Original) The method of claim 31, wherein the one or more selected cells are cells that produce a high level of product relative to other cells of the plurality.

33. (Currently amended) The method of claim ~~31~~, wherein the one or more selected cells are cells that produce a low level of product relative to other cells of the plurality.

34. (Original) The method of claim 31, wherein the one or more selected cells are cells that produce an undetectable level of product.

35. (Original) The method of claim 23, further comprising allowing the purified one or more selected cells to proliferate.

36. (Currently amended) The method of claim 23, wherein steps (a) through ~~[(d)]~~(e) are automated.

37. (Currently amended) The method of any of claims 23, 27 or 31, wherein the frame is a portion of a field-of-view, the field-of-view having an area selected ~~from the group of greater than 5 mm², greater than 10 mm², greater than 20 mm², greater than 40 mm², greater than 80 mm², and greater than 160 mm².~~

38. (Original) The method of claim 23, wherein the one or more selected cells are identified with reference to a signal value corresponding to an amount of product localized to the capture matrix in the vicinity of the one or more selected cells.

39. (Original) The method of claim 23, wherein the substantially lethal dose of radiation is delivered to each non-selected cell in one or more radiation pulses.

40. (Currently amended) The method of claim 23, wherein the substantially lethal dose of radiation has an energy density selected from the group consisting of greater than 0.1 J/cm², greater than 0.3 J/cm², greater than 1 J/cm², greater than 3 J/cm², greater than 10 J/cm², greater than 30 J/cm², and greater than 100 J/cm².

41. (Currently amended) The method of claim 23, wherein the substantially lethal dose of radiation has an irradiance selected from the group consisting of greater than 10⁷ W/cm²,

Appl. No. : 10/801,931
Filed : March 15, 2004

greater than 10^8 W/cm², greater than 10^9 W/cm², greater than 10^{10} W/cm², and greater than 10^{11} W/cm².

42. (Currently amended) The method of claim 23, wherein the radiation comprises an electromagnetic radiation having a wavelength selected from the group consisting of between 200 and 400 nm, between 400 and 760 nm, and between 760 and 3000 nm.

43. (Original) The method of claim 23, wherein prior to illuminating, the plurality of cells is contacted with a reagent that binds to substantially all of the cells, the reagent capable of generating a signal detectable as a first property of light.

44. (Currently amended) A method for purifying one or more selected cells in a population of cells, comprising:

(a) illuminating a population of cells in a frame, wherein the illuminated cells are contained in a plurality of cells immobilized in proximity to a capture matrix, wherein the capture matrix capable of localizing localizes a product secreted by one or more of the cells;

(b) detecting at least one property of light directed from the frame, wherein [[a]] the at least one property of light identifies product localized to the capture matrix;

(c) locating and selecting (i) one or more ~~selected~~ cells with reference to the detected property of light, and (ii) one or more domains in the frame, each domain corresponding to an area occupied by the one or more ~~at least one~~ selected cells, wherein the one or more domains are located with reference to the detected property of light;

(d) irradiating the non-domain area contained in the frame, wherein substantially all cells present within the non-domain area receive a substantially lethal dose of radiation, whereby the one or more selected cells having a desired product secretion profile are purified.

45. (Currently amended) The method of claim 44, wherein the product is selected from the group consisting of a polypeptide, an antibody, an antibody fragment, a cytokine, a growth factor, an enzyme, a hormone, a neurotransmitter, a signaling molecule, and a therapeutic protein.

Appl. No. : 10/801,931
Filed : March 15, 2004

46. (Currently amended) The method of claim 44, wherein the capture matrix comprises a substance selected from the group consisting of Protein G, Protein A, an antibody, an antibody fragment, an aptamer, and a ligand for the product.

47. (Original) The method of claim 44, wherein the capture matrix comprises a gel.

48. (Original) The method of claim 44, further comprising the step of (e) illuminating a further population of the cells, the further population contained in a further frame, and repeating steps (b) through (d).

49. (Currently amended) The method of claim 44 ~~or 48~~, wherein the one or more selected cells comprise a product secretion profile characterized by producing ~~are cells that produce~~ a high level of product relative to other cells of the population.

50. (Currently amended) The method of claim 44 ~~or 48~~, wherein the one or more selected cells comprise a product secretion profile characterized by producing ~~are cells that produce~~ a low level of product relative to other cells of the population.

51. (Currently amended) The method of claim 44 ~~or 48~~, wherein the one or more selected cells are cells that produce an undetectable level of product relative to other cells of the population.

52. (Original) The method of claim 48, further comprising repeating step (e) followed by steps (b) through (d) until substantially all of the non-selected cells in the plurality of cells receive a substantially lethal dose of radiation.

53. (Original) The method of claim 52, wherein the one or more selected cells are cells that produce a high level of product relative to other cells of the plurality.

54. (Original) The method of claim 52, wherein the one or more selected cells are cells that produce a low level of product relative to other cells of the plurality.

55. (Original) The method of claim 52, wherein the one or more selected cells are cells that produce an undetectable level of product relative to other cells of the plurality.

56. (Original) The method of claim 44, further comprising allowing the purified one or more selected cells to proliferate.

57. (Original) The method of claim 44, wherein steps (a) through (d) are automated.

Appl. No. : 10/801,931
Filed : March 15, 2004

58. (Currently amended) The method of any of claims 44, 48 or 52, wherein the frame is a portion of a field-of-view, the field-of-view having an area ~~selected from the group of greater than 5 mm², greater than 10 mm², greater than 20 mm², greater than 40 mm², greater than 80 mm², and greater than 160 mm².~~

59. (Original) The method of claim 44, wherein the one or more selected cells are identified with reference to a signal value corresponding to an amount of product localized to the capture matrix in the vicinity of the one or more selected cells.

60. (Original) The method of claim 44, wherein the substantially lethal dose of radiation is delivered in one or more radiation pulses to each cell present within the non-domain area.

61. (Currently amended) The method of claim 44, wherein the substantially lethal dose of radiation has an energy density selected from the group consisting of greater than 0.1 J/cm², greater than 0.3 J/cm², greater than 1 J/cm², greater than 3 J/cm², greater than 10 J/cm², greater than 30 J/cm², and greater than 100 J/cm².

62. (Currently amended) The method of claim 44, wherein the substantially lethal dose of radiation has an irradiance selected from the group consisting of greater than 10⁷ W/cm², greater than 10⁸ W/cm², greater than 10⁹ W/cm², greater than 10¹⁰ W/cm², and greater than 10¹¹ W/cm².

63. (Currently amended) The method of claim 44, wherein the radiation comprises an electromagnetic radiation having a wavelength selected from the group consisting of between 200 and 400 nm, between 400 and 760 nm, and between 760 and 3000 nm.

64. (Currently amended) A method for purifying one or more selected cells in a population of cells, comprising:

(a) contacting a plurality of cells immobilized in proximity to a capture matrix, wherein the capture matrix capable of localizing localizes a product secreted by one or more of the plurality of cells, with an agent that selectively binds to the product, wherein the agent capable of generating generates a signal detectable as a property of light;

(b) illuminating a population of the plurality of cells, the population contained in a frame;

Appl. No. : 10/801,931
Filed : March 15, 2004

(c) detecting at least one property of light directed from the frame, wherein a property of light identifies product localized to the capture matrix;

(d) locating and selecting ~~(i)~~ one or more selected cells with reference to the detected property of light, ~~and (ii)~~

~~(e)~~ selecting one or more domains in the frame, each domain corresponding to an area occupied by at least one selected cell, wherein the one or more domains are located with reference to the detected property of light, and

~~[[e]]~~ ~~(f)~~ irradiating the one or more non-domain areas contained in the frame, wherein each cell present within the non-domain areas receives a substantially lethal dose of radiation,

whereby one or more selected cells having a desired product secretion profile are purified.

65. (Currently amended) The method of claim 64, wherein the product is selected from the group consisting of a polypeptide, an antibody, an antibody fragment, a cytokine, a growth factor, an enzyme, a hormone, a neurotransmitter, a signaling molecule, and a therapeutic protein.

66. (Currently amended) The method of claim 64, wherein the capture matrix comprises a substance selected from the group consisting of Protein G, Protein A, an antibody, an antibody fragment, an aptamer, and a ligand for the product.

67. (Original) The method of claim 64, wherein the capture matrix comprises a gel.

68. (Currently amended) The method of claim 64, further comprising the step of ~~[[f]]~~ ~~(g)~~ illuminating a further population of the cells, the further population contained in a further frame, and repeating steps (c) through ~~[[e]]~~ ~~(f)~~.

69. (Currently amended) The method of claim 64 ~~or 68~~, wherein the one or more selected cells are cells that produce a high level of product relative to other cells of the population.

70. (Currently amended) The method of claim 64 ~~or 68~~, wherein the one or more selected cells are cells that produce a low level of product relative to other cells of the population.

Appl. No. : 10/801,931
Filed : March 15, 2004

71. (Currently amended) The method of claim 64 ~~or 68~~, wherein the one or more selected cells are cells that produce an undetectable level of product relative to other cells of the population.

72. (Currently amended) The method of claim 68, further comprising repeating step ~~[[f]]~~~~(g)~~ followed by steps (c) through ~~[[e]]~~~~(f)~~ until substantially all of the non-selected cells in the plurality of cells receive a substantially lethal dose of radiation.

73. (Original) The method of claim 72, wherein the one or more selected cells are cells that produce a high level of product relative to other cells of the plurality.

74. (Original) The method of claim 72, wherein the one or more selected product-secreting cells are cells that produce a low level of product relative to other cells of the plurality.

75. (Original) The method of claim 72, wherein the one or more selected cells are cells that produce an undetectable level of product relative to other cells of the plurality.

76. (Original) The method of claim 64, further comprising allowing the purified one or more selected cells to proliferate.

77. (Currently amended) The method of claim 64, wherein steps (b) through ~~[[e]]~~~~(f)~~ are automated.

78. (Currently amended) The method of any of claims 64, 68 or 72, wherein the frame is a portion of a field-of-view, the field-of-view having an area ~~selected from the group of greater than 5 mm², greater than 10 mm², greater than 20 mm², greater than 40 mm², greater than 80 mm², and greater than 160 mm².~~

79. (Currently amended) The method of claim 64, wherein the agent that selectively binds to the product is selected from the group consisting of an antibody, an antibody fragment, an aptamer, a substrate, and a ligand.

80. (Original) The method of claim 64, wherein the one or more selected cells are identified with reference to a signal value corresponding to an amount of product localized to the capture matrix in the vicinity of the one or more selected cells.

81. (Original) The method of claim 64, wherein the substantially lethal dose of radiation is delivered in one or more radiation pulses to each cell present within the non-domain area.

Appl. No. : 10/801,931
Filed : March 15, 2004

82. (Currently amended) The method of claim 64, wherein the substantially lethal dose of radiation has an energy density selected from the group consisting of greater than 0.1 J/cm², greater than 0.3 J/cm², greater than 1 J/cm², greater than 3 J/cm², greater than 10 J/cm², greater than 30 J/cm², and greater than 100 J/cm².

83. (Currently amended) The method of claim 64, wherein the substantially lethal dose of radiation has an irradiance selected from the group consisting of greater than 10⁷ W/cm², greater than 10⁸ W/cm², greater than 10⁹ W/cm², greater than 10¹⁰ W/cm², and greater than 10¹¹ W/cm².

84. (Original) The method of claim 64, wherein the radiation comprises an electromagnetic radiation having a wavelength selected from the group of between 200 and 400 nm, between 400 and 760 nm, and between 760 and 3000 nm.

85. (Currently amended) A method for determining the product secretion profile of one or more cells in a population of cells, comprising:

(a) illuminating a population of cells in a frame, wherein the illuminated cells are contained in a plurality of cells immobilized in proximity to a capture matrix, wherein the capture matrix localizes ~~capable of localizing~~ a product secreted by one or more of the cells;

(b) detecting at least one property of light directed from the frame, wherein a property of light identifies product localized to the capture matrix;

(c) locating one or more cells with reference to the detected property of light, and;

(d) determining a quantitative signal value from the property of light indicative of the product secretion profile of the one ~~or~~ or more cells.

86. (Currently amended) A method for determining the product secretion profile of one or more cells in a population of cells, comprising:

(a) contacting a plurality of cells immobilized in proximity to a capture matrix, wherein the capture matrix ~~capable of localizing~~ localizes a product secreted by one or more of the cells, with an agent that selectively binds to the product, wherein the agent ~~capable of generating~~ generates a signal detectable as a property of light;

Appl. No. : 10/801,931
Filed : March 15, 2004

(b) illuminating a population of the cells, the population contained in a frame, wherein the illuminated population is contained within the plurality of cells;

(c) detecting at least one property of light directed from the frame, wherein a property of light identifies product localized to the capture matrix;

(d) locating one or more cells with reference to the detected property of light, and;

(e) determining a quantitative signal value from the property of light indicative of the a product secretion profile.

87. (Withdrawn)

88. (New) The method of Claim 85 or 86, wherein the product is an antibody.

89. (New) The method of Claim 88, wherein the quantitative signal value is used to select cells for purification.

90. (New) The method of Claim 89, wherein the selected cells produce high-levels of recombinant humanized antibody relative to other cells in the population.

91. (New) A method for purifying one or more selected cells in a population of cells, comprising:

providing a population of cells immobilized in proximity to a capture matrix, wherein the capture matrix localizes a product secreted by one or more of the cells;

contacting the population of cells with an agent that selectively binds to the product, wherein the agent generates a signal detectable as a property of light;

illuminating the population of the cells;

detecting two or more properties of light, wherein a first property of light identifies substantially all of the population of cells, and a second property of light identifies a product localized to the capture matrix;

locating substantially all of the population of cells with reference to the detected first property of light;

selecting one or more cells from the population of cells with reference to the detected second property of light; and

irradiating a non-selected cell, wherein the non-selected cell receives a substantially lethal dose of radiation,

Appl. No. : **10/801,931**
Filed : **March 15, 2004**

whereby the one or more selected cells having a desired product secretion profile are purified.